In situ hybridization with oligonucleotide probes

Labeled oligonucleotides (30-50mers) can be used to detect RNAs. This method is particularly suited for the analysis of spliced vs. unspliced RNA (16, 17). The efficiency of detection is generally lower than with nick-translated probes.

Equipment and reagents

- Glass coverslips, 22x22mm
- 6-well plates
- 4% freshly made paraformaldehyde (Electron Microscopy Sciences) in PBS, pH 7.4
- Triton X-100 (Sigma)
- Parafilm
- Forceps, Dumont, GG (Electron Microscopy Sciences)
- Avidin-DCS-Texas Red or –fluorescein (Vector)
- Filterpaper
- Mounting medium (Molecular Probes)
- Microscopy coverslide
- Deionized formamide (Ambion)
- Yeast tRNA (10mg/ml) (Sigma)

Method

1. Cells grown on glass coverslips are fixed in 4% paraformaldehyde in PBS for 20 min at room temperature[a]
2. Wash the cells with PBS three times for 5 min each at room temperature
3. Permeabilize the cells with 0.2 % Triton X-100 in PBS on ice for 5 min
4. Wash the cells with PBS three times for 5 min each at room temperature
5. Wash the cell with 2x SSC for 5 min at room temperature
6. Add the following to a 1.5 ml microcentrifuge tube at room temperature 4 μl 20x SSC, 4 μl 50% dextran sulfate, ~ 1 μg/μl yeast tRNA, 0.2-1 pmol/μl

labeled oligonucleotide probe. Enough nuclease-free water to make a total reaction volume of 20 μl[a]

7. Place 20 μl hybridization mixture onto each coverslip and seal with rubber cement[a]
8. Put the slide into a chamber moistened with 2x SSC and incubate for at least 2-4 h at 37-42ºC
9. After hybridization, remove coverslips and wash three times in 4x SSC/0.1% Tween 20 for 5 min each at room temperature
10. Block each coverslip in 250 μl 4x SSC/3% BSA/0.1% Tween 20 for 20 min at room temperature
11. Incubate with avidin-conjugated with fluorochrome (2 μg/ml) in 4x SSC for 20 min at room temperature
12. Wash three times in 4x SSC/0.1% Tween 20 for 5 min each at 37ºC
13. Mount the coverslip into mounting media[c]

[a] The following recipe is for a volume 20 μl, which is sufficient for one hybridization reaction covering an area of 22 x 22 mm

[b]Hybridization without labeled probe should be performed as a control with each experiment

[c]When in situ hybridization is followed by immunofluorescence, after rinsing of cells in PBS, incubate the cells with primary antibody, then rinsed in PBS and incubate with appropriate secondary antibody

- FISH is compatible with detection of proteins by indirect immunofluorescence. Perform the IF first and then proceed to the FISH.